As an initial matter, it is noted that the Examiner has mischaracterized the claimed invention as being directed to a method for producing *conifer* somatic embryos. Rather, it is noted that the pending claims are directed to a method of producing a synchronized population of *pine* somatic embryos, as shown below in Claim 1:

> A method for producing a synchronized population of *pine* somatic embryos, the method comprising:
>
> (a) cultivating pre-cotyledonary *pine* embryogenic cells in, or on a maintenance medium comprising nutrients that sustain the pine embryogenic cells, wherein the osmolality of the maintenance medium is from 180 mM/Kg to 400 mM/Kg;
>
> (b) cultivating pre-cotyledonary *pine* embryogenic cells from step (a) for a period from 0.5 weeks to 5 weeks in, or on, a synchronization medium that comprises an absorbent composition and at least one synchronization agent selected from the group consisting of abscisic acid and a gibberellin, wherein the absorbent composition and the at least one synchronization agent are present at a concentration effective to produce a synchronized population of pre-cotyledonary pine somatic embryos wherein at least 50% of the pre-cotyledonary pine somatic embryos in the synchronized population are at the same developmental stage; and
>
> (c) transferring the synchronized population of pre-cotyledonary *pine* somatic embryos from step (b) to a development medium and culturing the pre-cotyledonary pine somatic embryos for a period from 9 to 14 weeks to produce a synchronized population of cotyledonary pine somatic embryos. [Emphasis added.]

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Pullman et al. does not teach or suggest the claimed invention for at least the following reasons. Contrary to the Examiner's assertion, Pullman et al. does not disclose the step of singulation for Loblolly pine. The Examiner characterizes Pullman et al. as disclosing transferring embryos to a singulation medium comprising gibberelin and/or abscisic acid at concentrations of 0.05 and 15g/L (with reference to Col. 13, lines 40-60) and comprising also activated charcoal (Col. 13, lines 50-54) for at least 3 weeks (Col. 15, lines 23-26). However, it is noted that the passages relied upon by the Examiner describe the singulation stage used to culture *Douglas fir* somatic embryos. There is no teaching or suggestion in Pullman et al. to culture *pine* embryos in the multistep process as recited in Claim 1, with a first incubation on maintenance media, followed by incubation in synchronization medium, followed by incubation in development media. Rather, Pullman et al. actually teaches away from the use of the singulation step during embryo culture for species other than Douglas fir, such as pine, by stating, "Douglas-fir generally requires an intermediate step between the late proembryo growth stage and the final cotyledonary embryo development stage which is not necessary for other species. The proembryos tend to form in tight clumps or clusters which must first be singulated before going to the development stage." Pullman et al., at Col. 8, lines 18-23 (emphasis added). Consistent with the teaching in Pullman et al. regarding the need for singulation for culturing embryos from Douglas fir and not from other species, Examples 1, 2, 3, 4, 5, 6, and 7 of Pullman et al., which are all directed to methods of growth of Douglas fir embryos, all include the singulation step (e.g., see Col. 14, line 4, to Col. 20, line 40); whereas the two examples in Pullman et al. directed to methods for culturing embryos from species other than Douglas-fir (e.g., Norway spruce in Examples 8 and 9) both describe plating from a maintenance medium directly onto solid development medium, with no singulation step. See Pullman et al. at Col. 20, line 41, to Col. 23, line 30.

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Moreover, Pullman et al. does not remotely teach, suggest, or provide any motivation to produce a synthesized population of cotyledonary pine somatic embryos by incubation in a synchronization medium prior to incubation in development media, *wherein at least 50% of the pre-cotyledonary pine somatic embryos in the synchronized population are at the same developmental stage* ***prior*** *to transfer to a development medium,* as recited in Claim 1.

As described in the instant specification:

> Cleavage polyembryony (embryonal suspensor mass proliferation) continues in cultures after plating onto development medium, and new embryos are beginning to develop even after eight to ten weeks of culture on development medium. Due to this continuing cleavage, embryos are not uniform in stage, shape, size, or quality within a single plate. This lack of uniformity detrimentally affects the efficiency of somatic cloning of conifers. The present invention addresses the problem of unsynchronized development of conifer embryogenic cells, including ESMs, by culturing the embryonic cells in, or on, a synchronization medium that causes the majority of embryos in a population of conifer somatic embryos to progress through successive developmental stages together to yield a synchronized population of mature conifer somatic embryos that can be germinated to form conifer plants. [Specification at page 4, lines 18-28.]

There is no disclosure in Pullman et al. regarding synchronization of embryo growth. As noted above, Pullman et al. teaches culturing Douglas fir embryos in singulation medium prior to development medium and teaches away from singulation for other species, such as pine. Rather, as stated in Pullman et al. at Col. 7, lines 12-14, "It should be noted here that Douglas-fir does not experience polyembryony as do most other coniferous species."

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Therefore, it is demonstrated that the incubation of *pine* embryos in synchronization media for 0.5 to 5 weeks *prior* to incubation in a development media as recited in Claim 1 step (b) is an important distinction between the Pullman et al. reference and the present invention.

With regard to the Examiner's response to applicants' previous arguments, it is noted that the Examiner is incorrect in the assertion that Pullman et al. states that adding the singulation step is beneficial for improvement of proembryo quality, with reference to Col. 8, lines 5-14. Rather, as noted above, Pullman et al. actually teaches away from the use of the singulation step during embryo culture for species other than Douglas fir, such as pine, by stating, "Douglas-fir generally requires an intermediate step between the late proembryo growth stage and the final cotyledonary embryo development stage which is not necessary for other species."

With regard to the Examiner's assertion that Pullman et al. teaches that this method can be used for many species, including loblolly pine (with reference to Col. 7, lines 50-60), it is noted that the passage of Pullman referred to by the Examiner describes *development medium*. It is noted that the claimed invention is directed to the incubation of pine embryos in synchronization media for 0.5 to 5 weeks *prior to* incubation in a development media.

The Examiner further asserts that Pullman et al. anticipates the claimed method because Pullman et al. states, "[s]pecies other than Douglas-fir can be advantageously cultured by beginning early cotyledonary embryo development in a series of media similar to those used for Douglas-fir singulation." It is again noted that step (b) of the claimed method is directed to incubation in synchronization media for 0.5 to 5 weeks *prior to* incubation in a development media.

Accordingly, it is demonstrated that Pullman et al. does not teach or suggest the method of producing a synchronized population of *pine* somatic embryos, as claimed. Further, Pullman

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et al. does not even remotely address the unexpected result obtained by the claimed invention. As described in Examples 1 and 2 of the instant specification, the present inventors discovered through experimentation that culturing pine embryos in synchronization medium containing activated charcoal and at least one of abscisic acid and a gibberellin prior to incubation in development media inhibited precocious embryo development and greening, while promoting singulation and synchronization of the cultures, resulting in embryos very uniform in size in comparison to control cultures. See specification at page 19, lines 19-31. Thus, without the benefit of applicants' disclosure, one of skill in the art would not be motivated by the teachings of Pullman et al., or by the general knowledge in the art, to arrive at the claimed invention.

Accordingly, applicants respectfully submit that the claimed invention is clearly patentable over Pullman et al.

Conclusion

Applicants believe that Claims 1-13, 15-19, 21, 23, and 24 are in condition for allowance. Reconsideration and favorable action is requested. If any issues remain that may be expeditiously addressed in a telephone interview, the Examiner is encouraged to telephone applicants' attorney at 206.695.1655.

Respectfully submitted,

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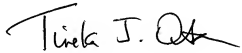

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